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DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Request for Information: The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods Requests the Nomination of Reference Chemicals.

SUMMARY: The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) requests the nomination of reference chemicals, with supporting data, to be used to validate in vitro metabolizing systems with the potential to interact with estrogen receptors (ERs) or androgen receptors (ARs). Specifically, a list of chemicals is needed to characterize the usefulness and limitations of in vitro metabolizing systems for use in conjunction with ER and AR transactivation tests.

DATES: The deadline for receipt of information is June 2, 2014.

ADDRESSES: Nominated reference chemicals and associated data should be submitted electronically in Microsoft® Excel or Word formats to niceatm@niehs.nih.gov. A Microsoft® Excel template for data submission is available at http://ntp.niehs.nih.gov/go/41493.

FOR FURTHER INFORMATION CONTACT: Dr. Warren S. Casey, Director, NICEATM; email: warren.casey@nih.gov; telephone: (919) 316-4729.

SUPPLEMENTARY INFORMATION:

Background: Endocrine-active substances (EAS) are chemicals that interfere with normal endocrine hormone function by mimicking, blocking, or increasing their actions, thereby possibly causing adverse health effects. United States legislation (e.g., 7 U.S.C. 136, 110 Stat 1613) requires that chemicals be tested for their ability to disrupt the hormonal systems of mammals; prospective international legislative proposals may have similar requirements. Chemicals found to test positive in vitro as EAS may be in vivo endocrine disruptors. The lack of in vitro tools that mimic in vivo metabolism is the main obstacle to implementation of in vitro tools for EAS toxicity testing. Improved understanding of metabolic capabilities and limitations of in vitro toxicity testing is critical to:

- Ensure that no potentially active metabolites are missed
- Allow better interpretation of results
- Accurately predict species-specific characteristics of absorption, metabolism, and excretion

While there is a growing body of international in vitro test guidelines addressing EAS mechanisms and modes of action, there are few or no standardized methods to incorporate metabolic and toxicokinetic aspects into these EAS in vitro tests to date. In vitro assays for EAS should incorporate metabolic enzyme systems to better address the

relevance of EAS tests to in vivo adverse outcome pathways.

The Organization for Economic Co-operation and Development (OECD) Validation Management Group-Non-Animal (VMG-NA) expert working group develops internationally accepted non-animal test guidelines to support various international regulatory needs for the hazard identification of potential EAS. These test guidelines describe methods and approaches capable of identifying potential EAS without the use of animals. Consistent with its purpose of evaluating alternative methods for testing chemicals and chemical products, NICEATM participates in the VMG-NA.

Test guidelines for in vitro assays for ER activity have been evaluated and accepted by international regulatory authorities; test guidelines for in vitro AR activity assays are currently in development. However, none of these in vitro EAS assays account for whole animal metabolism. Further development of specific tests is needed to optimize the use of in vitro metabolism with EAS assays. Identification of appropriate reference chemicals to check the metabolic capacity of any proposed test method is key to continued assay development. For this purpose, the VMG-NA is developing a robust list of chemicals that, when metabolized, act as ER or AR agonist or antagonists.

Request for Information: On behalf of the VMG-NA, NICEATM requests nominations of chemicals that can be used to characterize and validate in vitro metabolizing systems for use in conjunction with in vitro tests for ER and AR transactivation. Responses are requested from all interested parties, including the research community, health professionals, educators, policy makers, industry, and the public. Considerations for selection of appropriate chemicals include the ability of a chemical to act as an ER or AR

agonist or antagonist and:

- Potential for metabolism to make a chemical either more potent (bioactivation) or less potent (detoxification)
- Likelihood of metabolism occurring in relevant routes of exposure and target organs
- Likelihood of metabolism occurring over a range of doses: information on the ratio of the half maximal effective or inhibitory concentration (EC50 or IC50, respectively) of parent to daughter metabolites will be useful and there is a particular need for information pertaining to substances where biotransformation yields a very small or very large ratio of EC50/IC50 of parent to daughter metabolites
- Stability, preferably with real-time curves and consequent exposure significance of likely metabolites
- Diversity of likely and predominant biotransformative pathways
- Diversity of chemical types, use classes, and consequent applicability domains

The reference chemicals will be used to check the metabolic capacity of the in vitro model, including characterization of the general metabolic capacity of the cell lines. To ensure relevant use in a regulatory context, it will be necessary, where possible, to make correlations to:

(a) Relevant in vivo metabolic modeling (accounting for absorption, distribution, metabolism, and excretion, etc.) of plasma/blood metabolites in vertebrate animals (e.g., rat, fish, human).

(b) Data from the uterotrophic, Hershberger, and/or other relevant assays with a

demonstrated high confidence in prediction of bioactivation of estrogenic or androgenic agonist and antagonist pathways, such that the true systemic in vivo metabolic response is addressed as accurately as possible.

When reporting the in vitro dose response for potential reference chemicals, the concentrations of solvent and/or carrier proteins used in the assay buffers to solubilize the reference chemicals should be described to facilitate an understanding of potential differences among new in vitro assays with regard to free concentrations of parent chemical and metabolites versus nominal dosages within each testing system.

Nominated reference chemicals and associated data should be submitted electronically in Microsoft® Excel or Word formats to niceatm@niehs.nih.gov. Data submitted can include, but need not be limited to, citations of reports in the published literature, data from past or ongoing validation studies, data in databases, or unpublished data. A template for data submission is available at http://ntp.niehs.nih.gov/go/41493.

Responses to this request are voluntary. NICEATM does not intend to publish a summary of responses received or any other information provided. No proprietary, classified, confidential, or sensitive information should be included in your response. Please note that the U.S. Government will not pay for the preparation of any information submitted or for its use of that information.

Those submitting information should include name, affiliation, mailing address, phone, fax, email address, and sponsoring organization (if any) with the submission. The deadline for receipt of the requested information is June 2, 2014.

Background Information on NICEATM: NICEATM conducts data analyses,

workshops, independent validation studies, and other activities to assess new, revised, and alternative test methods and strategies and provides support for the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*–3) provides authority for ICCVAM and NICEATM to conduct activities relevant to the development of alternative test methods. Information about NICEATM and ICCVAM is found at http://ntp.niehs.nih.gov/go/niceatm and http://ntp.niehs.nih.gov/go/iccvam.

Dated: May 7, 2014.

John R. Bucher,

Associate Director, National Toxicology Program.

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